United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine

Soil and Potting Medium Sampling and Baiting for *Phytophthora ramorum* in Infested or Potentially Infested Nurseries

Adapted from protocols in use by the Oregon Department of Agriculture.
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Soil and Potting Media Sampling

Infested soil or potting media will look exactly the same as un-infested soil or potting media. Therefore all soil and media must be handled carefully. All tools used to collect soil or media samples must be disinfected with 10% bleach solution, 70% ethanol, or flame-sterilized with a propane torch. All soil and organic material should be removed from the tools prior to disinfection. Care should also be taken not to transfer soil or potting media from one block to the next on shoes or clothing. All sampling equipment should be cleaned and disinfected prior to entering a new nursery block. Care must be taken to ensure that un-infested soil or potting media is not contaminated by infested soil or potting media.

Preparing for Sampling

Soil samples should be collected as composite samples. Each composite sample should contain a maximum of 500-ml (volume) of soil and/or potting media. The number of composite samples collected will depend upon the size of the nursery block (see Table 1).

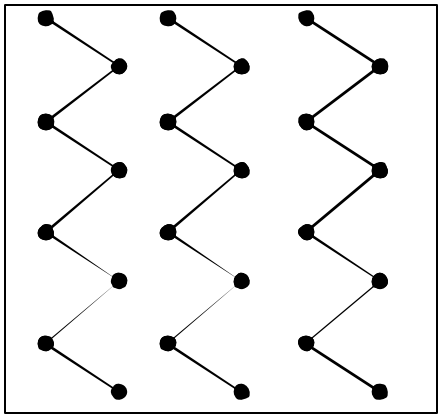
Table 1. Number of composite samples collected based on nursery block size.

Size of Treated Site (acres)	No. of Soil Samples Collected
0.00 - 0.25	1
0.26 - 0.5	2
0.50 - 1.0	4
1.01 - 2.50	8
>2.51	12

Each composite sample will consist of a minimum of five sub-samples collected from soil and/or potting media within the targeted area. Sub-samples should be collected according the pattern in the diagram below (Figure 1). Alternatively, if fallen leaves or other debris from the infected plants are present; sub-sampling may be targeted towards those areas. The location of each composite sample should be recorded (preferably by GPS) in case follow-up treatment of the soil/potting media for P. ramorum is required. Composite samples may also be collected from neighboring blocks of un-infested plants using the same steps. If you are collecting from blocks of un-infested plants, collect the composite soil/potting media samples from these blocks first to minimize the risk of contaminating un-infested soil/potting media. If all potentially-infested

potting media has been destroyed with the infected plants, collect composite samples from the remaining host plants within 2- to 10-m of the originally infected plants that have been placed on hold. Preferentially target the potting media of those plants that are “downstream” (e.g., based on watering patterns) of the originally infected plants.

Figure 1. Recommended pattern for collection of sub-samples for composite soil and/or potting media samples.



Soil Baiting

When the soil samples arrive, place the sample submission form in the appropriate binder. Record the sample number, "soil" and nearest host, date collected, and date processed in the appropriate laboratory notebook. Enter the information from the sample submission form into the appropriate SOD post-treatment monitoring survey database. Results of laboratory isolation will be entered into the same database.

To prepare soil bait, briefly soak the pears (select unripe green pears), Rhododendron leaves or Viburnum leaves in a mild detergent solution to remove any pesticide residues. Rinse the baits well and drain.

Leaving the soil in the Ziploc bag, add enough sterile deionized water to saturate and cover soil with about 2.5 cm (1") of water. Do not mix the soil and water.

Use two pears or leaves per soil sample. With a black sharpie pen, label one side of the pears or leaves with the soil sample number and date processed. If using Rhododendron or Viburnum leaves, the leaves should be wounded by trimming off the petioles prior to being placed in the soil/water mixture (E.M. Hansen, OSU, personal communication).

Carefully push each pear or leaf into the wet soil and water until the bait is immersed halfway. Leave the labeled side of the bait out of the water. Seal the Ziploc bag and leave bait in the soil/water mixture for at least 48-hr at room temperature (RT).

After 48-hr, remove the baits and wash off any clinging soil into Ziploc bag. Set the bait on a moistened paper towel in a sealed container at RT for 7-d to let any potential disease symptoms develop. The soil/water mixture must be autoclaved before disposal.

Examine the bait daily for developing symptoms. Pears infected with *P. ramorum* will display lesions that are round, brown, somewhat leathery in texture, with undefined edges. Colorless, watery, and/or soft lesions are generally caused by other pathogens (especially *Pythium* spp.). Rhododendron or Viburnum leaves that have become infected with *P. ramorum* will exhibit 'diffuse' leaf spots usually with the midvein most affected.

Under the laminar flow hood, cut eight to 10 pieces of pear or leaf from the edge of the developing lesion or leaf spot and insert into the PARP medium. Write the sample number and date processed on the underside of the Petri dish. Seal the dish with parafilm and incubate and treat as described in the USDA approved *Guidelines for Isolation by Culture and Morphological Identification of Phytophthora ramorum* (*http://www.aphis.usda.gov/ppq/ispm/sod/cultureprotocol.html*).

This protocol, in one form or another, has been used for decades to isolate Phytophthora species from soil and/or water samples, however, this protocol has not been completely validated by PPQ and may be adjusted periodically as the validation process is undertaken

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